Role of apolipoprotein C-III overproduction in diabetic dyslipidemia

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ABSTRACT

Aims: ApoC-III is an important novel target underpinning the link between hypertriglyceridemia with cardiovascular disease. Here, we investigated how apoC-III metabolism is altered in subjects with type 2 diabetes, and focused on whether the perturbed plasma triglyceride concentrations in this condition are determined primarily by the secretion rate or the removal rate of this apoprotein. Second, we investigated whether improvement of glycemic control using the GLP-1 analogue liraglutide for 16 weeks modifies apoC-III dynamics.

Materials and methods: Postprandial apoC-III kinetics were assessed after a bolus injection of [5,5,5-$^{2}H_3$]leucine, using ultrasensitive mass spectrometry techniques. We compared apoC-III kinetics in two situations; in subjects with type 2 diabetes subjects before and after liraglutide therapy, and in type 2 diabetic and BMI-matched non-diabetic subjects. Liver fat content, subcutaneous abdominal and intra-abdominal fat were determined using proton magnetic resonance spectroscopy.

Results: Improved glycemic control by liraglutide therapy for 16 weeks reduced significantly apoC-III secretion rate (561±198 vs. 652±196 mg/day, p=0.03) and apoC-III levels (10.0±3.8 vs. 11.7±4.3 mg/dL, p=0.035) in type 2 diabetic subjects. Change in apoC-III secretion rate was associated significantly with the improvement in indices of glucose control (r=0.67; p=0.009) and change in triglyceride AUC (r=0.59; p=0.025). In line, the apoC-III secretion rate was higher in subjects with type 2 diabetes compared with BMI-matched non-diabetic subjects (676±208 vs. 505±174 mg/day, p=0.042).

Conclusions: The results reveal that the secretion rate of apoC-III associates with elevation of triglyceride-rich lipoproteins in type 2 diabetics, potentially through the influence of glucose homeostasis on the production of apoC-III.

Keywords: apoC-III; kinetics; lipoproteins; stable isotopes; type 2 diabetes

INTRODUCTION

Apolipoprotein C-III (apoC-III) is increasingly recognized as an important determinant of hypertriglyceridemia and a risk factor for cardiovascular disease (CVD)[1-4]. ApoC-III also substantially improves the prediction of CVD in type 2 diabetes beyond that obtained with the variables used in the Framingham risk score[5]. Loss of function mutations in the gene encoding apoC-III (APOC3) is associated with low triglyceride levels[6,7], and a decreased risk for CVD while overexpression of APOC3 is associated with hypertriglyceridemia[8]. These results have identified apoC-III as an emerging target linking hypertriglyceridemia with cardiovascular disease, and clinical trials on apoC-III antisense oligonucleotides designed to inhibit synthesis of the apoprotein are in progress[9].

ApoC-III is a small apoprotein that is mainly secreted from the liver on very low-density lipoprotein (VLDL) particles and to a lesser degree from the intestine on chylomicrons. In the circulation, apoC-III is continuously exchanged between different lipoprotein particles and significant amounts of apoC-III are carried not only on VLDL but also on low- and high-density lipoprotein (LDL and HDL) particles[10,11]. The distribution of apoC-III between different lipoprotein fractions is dependent on the metabolic state, varying between the fasting and postprandial states and between subjects with normal and high plasma triglyceride levels[11-14]. The impact of apoC-III on CVD risk is complex but in general, lipoproteins with high apoC-III seem to be highly atherogenic[2,15,16].

ApoC-III has multiple actions on lipid metabolism as well as on proinflammatory and atherogenic processes[12-14]. It is well known as an inhibitor of lipoprotein lipase (LPL), the rate-limiting enzyme in the lipolytic process[17-19], and more recent data indicate that it also impairs the uptake of lipoprotein remnants by the liver[20-23]. We have earlier reported that plasma triglyceride concentrations in abdominal obesity are determined by the kinetics of VLDL1 subspecies, catabolism being mainly dependent on apoC-III concentration [24]. In addition, overexpression of apoC-III has been reported to promote VLDL assembly and secretion in hepatocytes *in vitro* and in genetically modified mice[25,26]; however, it is not known if this also occurs in humans.

Promoter analysis of *APOC3* identified binding sites for transcription factors including the carbohydrate response element binding protein (ChREBP)[27], which led to the proposal that glucose and plasma triglyceride metabolism are linked via the regulation of *APOC3* expression[12]. *APOC3* expression is upregulated by glucose and downregulated by insulin, and it has been suggested that the glucose-mediated expression of *APOC3* may contribute to lower lipolysis and to an increase in peripheral glucose handling[27-30]. In addition, the inhibitory effect of insulin on *APOC3* expression is lost when insulin signaling is impaired, which may explain why apoC-III concentrations are high in

insulin-resistant states such as obesity, the metabolic syndrome, type 2 diabetes, and hypertriglyceridemia[15,27,31].

Glucagon-like peptide-1 (GLP-1) agonists have been shown to improve postprandial lipid metabolism.[32,33] In line, we reported that liraglutide therapy for 16 weeks improved multiple cardiometabolic risk factors, inducing a reduction in plasma apoC-III[34]. It was estimated, that the decrease in fasting and postprandial levels of apoC-III during liragutide therapy explained about 50% of the observed reduction in postprandial triglyceride, RLP-cholesterol and TRL-cholesterol. These observations prompted us to perform apoC-III kinetic studies before and after liraglutide therapy

In this study, we investigated for the first time how the dynamics of apoC-III metabolism are altered in subjects with type 2 diabetes, and focused on whether plasma triglyceride concentrations are determined by the secretion rate or the removal rate of apoC-III. Second, we investigated whether improvement of glycemic control using GLP-1 analogue liraglutide for 16 weeks modifies apoC-III dynamics.

METHODS

Study cohort The current study is an extension of a previously reported intervention protocol to examine the effects of the GLP-1 analogue liraglutide 1.8 g daily for 16 weeks on glycaemia, ectopic fat depots and cardiometabolic risk factors[34]. Details of the recruitment of subjects are previously reported[34]. Here we analyzed whether improved glycemic control in subjects with type 2 diabetes effects apoC-III kinetics. We included 14 obese men [waist >92 cm, body mass index (BMI) 27–40 kg/m^2] with type 2 diabetes who had HbA1c 42–75 mmol/l (6–9%) and who completed the intervention period with liraglutide. To explore the effect of type 2 diabetes on apoC-III kinetics, we were able to identify from our previous kinetic studies[35] 11 obese men without type 2 diabetes matched for age and BMI as non-diabetic comparators for 11 (out of the 14) subjects with type 2 diabetes. Additional inclusion criteria were age 30–75 years, triglycerides 1.0–4.0 mmol/l and LDL-cholesterol <4.5 mmol/l. All those with type 2 diabetes used stable daily doses of metformin (between 1.0–3.0 g/ day) and statin throughout the study period[34]. Exclusion criteria for all subjects were: age <30 years or >75 years, smoking, alcohol consumption over 2 units/day (*i.e.,* 20 g pure alcohol), cardiovascular disease, hormonal therapy, use of fibrates and fish oils, hepatic and renal diseases, eGFR <60 ml/min, gastroenterological, thyroid or hematological abnormalities, and any chronic disease requiring medication except for controlled hypertension. The study protocol was approved by the ethics committees of Helsinki University Hospital and the national Agency of Medicines, Helsinki, Finland (Eudra CT 2013-005075-40, Clinical Trials NCT 92765399). Each subject gave

written informed consent before participation in the study. All studies were performed in accordance with the Declaration of Helsinki for clinical trials.

Study design—The actual protocol included two separate visits within a 1-week period: (1) a postprandial apoC-III kinetic study using stable isotopes, and (2) determination of abdominal fat depots and liver fat content. These tests were repeated in the liraglutide intervention group after 16 weeks liraglutide treatment.

Postprandial kinetic study: The subjects were admitted at 7:30 am after an overnight fast and baseline blood samples were taken. At 8:00 am, a bolus injection of [5,5,5-$^{2}H_3$]leucine [7 mg/kg] was given and blood was drawn during the 2 h after the injection as described previously[36].Two hours after the bolus injection, the subjects received a fat-rich meal served with a cocoa-fat rich emulsion containing 40 g of olive oil (Amway, Firenze). The meal consisted of bread, cheese, ham, boiled eggs, fresh red pepper, low-fat (1%) milk, orange juice and tea or coffee. Altogether, the meal contained 63 g carbohydrate, 69 g fat and 40 g protein, and was consumed within 10 min. Blood samples were taken at 0.5, 1, 2, 3, 4, 6, 8 and 10 hrs. after the meal. Water was allowed *ad libitum* and the subjects remained physically inactive.

Determination of intra-abdominal fat depots: Proton magnetic resonance spectroscopy was performed using a 1.5-T whole-body device to determine liver fat content[37,38], as well as subcutaneous abdominal and intra-abdominal fat[39]. All analyses of the imaging results were performed by one person (AH). Subjects were advised to fast for 4 h before imaging.

Analysis of hepatic de novo lipogenesis (DNL)—The DNL analyzed at 0 h and was calculated from enrichment of deuterated water ingested during the kinetic study at the specified time points [40].

Isolation of lipoprotein fractions—Lipoprotein fractions [chylomicrons (Sf >400), large $VLDL_1$ particles (Sf 60–400) and smaller $VLDL_2$ particles (Sf 20–60)] from all serum samples drawn during the postprandial apoC-III kinetic study, were separated by density gradient ultracentrifugation[41].

Biochemical analysis—Triglyceride and cholesterol concentrations in total plasma and lipoprotein fractions as well as in triglyceride-rich lipoprotein (TRL) cholesterol and remnant-lipoprotein (RLP) cholesterol were analyzed using assays (Denka Seiken, Tokyo, Japan) and the Konelab 60i analyzer (Thermo Fisher, USA). RLP-C captures lipoproteins not binding with anti-apoA-I and anti-apoB-100 as remnant lipoproteins[42]. The TRL-C quantifies cholesterol in both chylomicron remnants, and VLDL-IDL particles. Fasting and postprandial apoB48 levels in total plasma were measured by ELISA (Shibayagi, Shibukawa, Japan). Concentrations of plasma glucose and insulin were measured with hexokinase method (Roche Diagnostic Gluco-quant, Germany) and electrochemiluminescence (Roche sandwich immunoassay on a Cobas autoanalyzer), respectively. Plasma levels of apoC-III

were measured immunoturbidometrically (Kamiya Biomedical Company, Seattle, WA) and β-hydroxybutyrate concentrations were measured by an enzymatic method with β-hydroxybutyrate FS kit (DiaSys Diagnostic Systems, Holzheim, Germany) on a Konelab 60i analyzer.

Calculation of estimated GFR (eGFR)—Estimated Glomerular filtration rate (eGFR) was estimated using the revised Lund-Malmö equations based on age, gender and creatinine[43].

Kinetic analysis—Tracer kinetics ([5,5,5-$^{2}H_3$]leucine) of apoC-III was modelled using an established model including a four compartment system for free leucine kinetics coupled to a delay compartment representing synthesis and secretion of apoC-III[44] (see details in on-line Supplementary information). The kinetic model was implemented as a population kinetic model in Monolix (version 2016R1. Antony, France: Lixoft SAS, 2018). All model parameters were assumed to be log-normally distributed. Individual parameters were calculated using conditional means using Monolix. Further details of the implementation are given in the on-line **Supplementary information**.

Statistical analysis—Statistical analyses were performed with R (R Foundation for Statistical Programming, www.r-project.org) version 3.2.1 and GraphPad Prism version 7 (La Jolla, CA). Data are presented in tables as mean ± SD and in figures as mean ± SE. We calculated the area under the curve (AUC) using the trapezoidal rule between 0 and 8 hours after the meal. Subjects with and without type 2 diabetes were compared using Mann-Whitney U-test, unless stated otherwise. P-values <0.05 were considered statistically significant and were not adjusted for multiple testing.

RESULTS

Effects of liraglutide therapy on apoC-III metabolism

We tested the hypothesis that improved glycemic control would affect apoC-III metabolism in type 2 diabetic subjects. We have recently reported effects of liraglutide treatment on glycemic control, fat depots as well as fasting and postprandial lipids in type 2 diabetic subjects[34]. Characteristics of these 14 individuals are summarized in **Suppl. Table S1**, and postprandial responses are summarized in **Suppl. Table S2**. Overall liraglutide therapy was associated with beneficial changes of atherogenic lipids/ lipoproteins and apoC-III level (**Suppl. Table 1** and **2**). Notably, apoC-III response (postprandial AUC) was reduced by 13% during liraglutide therapy. This raises the question what are the factors driving these changes. ApoC-III secretion rate and apoC-III plasma concentration, but not apoC-III fractional catabolic rate, correlated strongly with the plasma triglyceride concentration (**Suppl. Figure 1**). The data suggest that the secretion rate of apoC-III is a major determinant for the concentration of plasma triglycerides.

Treatment with liraglutide resulted in a significant decrease in fasting apoC-III concentration (-14%;

11.7±4.3 mg/ dl vs 10.0±3.8 mg/ dl, p<0.05). Examining the enrichment curves, it was observed that there was a similar decay rate for the tracer in apoC-III in both treatment periods (**Suppl. Figure S2**) and this was reflected in the lack of change in FCR (**Figure 1 and Table 1**). Accordingly, the decreased pool was attributable to a 13% decrease in apoC-III synthesis (6.6±1.7 mg/kg/day on placebo vs 5.8±2.0 mg/kg/day on liraglutide, p<0.05). Indeed, apoC-III SR turned out to be the major predictor of plasma apoC-III pool both before and after liraglutide therapy in these type 2 diabetic subjects (r>0.83; p<0.001). Further, changes of apoC-III secretion rate and apoC-III pool size correlated with those of plasma TG AUC (**Figure 2 E** and **F**).

It was also noteworthy that there were significant associations during liraglutide therapy, between changes in parameters of glycemic control and apoC-III SR (r=0.67; p=0.009) and pool-size (r=0.75; p=0.002) in the Type 2 diabetic subjects (**Figure 2**). In contrast, there was no significant association between changes in parameters of glycemic control and apoC-III FCR (r=0.40; p=0.15) (**Suppl. Figure 3**).

Effects of type 2 diabetes on apoC-III kinetics

To explore the effect of type 2 diabetes on apoC-III kinetics, we were able to identify from our previous kinetic studies[35] 11 obese men without type 2 diabetes matched for age and BMI as non-diabetic comparators for 11 (out of the 14) subjects with type 2 diabetes. Baseline data and biochemical measures of the 11 men with type 2 diabetes and the non-diabetic subjects are shown in **Table 2**. All were abdominally obese and the two groups were well matched for age, BMI and waist circumference. In the diabetic group duration of diabetes averaged 6.6 years while parameters of glycemic control (i.e., glucose and HbA1C) indicated that acceptable glycemic control had been achieved. Plasma triglyceride levels were not significantly higher in the men with type 2 diabetes. Total cholesterol, and LDL- and TRL-cholesterol concentrations were, as expected, lower in the men with type 2 diabetes since these subjects were all on statin therapy. The diabetic group had 2.6-fold higher liver fat content and 3.4-fold higher DNL than in the non-diabetic subjects; β-hydroxybutyrate, a surrogate marker of hepatic β-oxidation, was similar in the two groups (**Table 2**).

Postprandial triglyceride responses to the test meal in plasma, chylomicrons, $VLDL_1$ and $VLDL_2$ fractions were analyzed in the two groups (**Suppl. Figure S4**). Despite comparable triglyceride levels at baseline and at early time points after the meal in the two groups, the triglyceride values 8 h after the meal were markedly higher in the group with type 2 diabetes. Plasma fasting apoC-III showed a wide range in both groups (non-diabetic comparator group, 2.8–13.7 mg/dl; type 2 diabetes, 3.2–17.7 mg/dl), and postprandial apoC-III concentrations showed a non-significant trend to be higher in the group with type 2 diabetes (**Suppl. Figure S4**).

Kinetic parameters (secretion rate and fractional catabolic rate) for plasma apoC-III (**Table 1**) were compared in the 2 groups. During the evaluation period apoC-III levels were in steady state (**Suppl. Figure S4**). Mean tracer enrichment curves exhibited a steeper rise and fall in the diabetic group (**Suppl. Figure S2**) than in non-diabetic comparator group, and this translated into a 39% higher apoC-III secretion rate and a 17% higher fractional catabolic rate in the former versus the latter subjects.

Consequently, no significant difference was observed in the model-predicted apoC-III pool (**Table 1**). A strong positive correlation between the apoC-III secretion rate and circulating apoC-III pool existed across all subjects but there was no significant correlation between the apoC-III fractional catabolic rate and the apoC-III pool (**Suppl. Figure 5**). These results indicate that apoC-III secretion rate is an important determinant of plasma apoC-III concentration.

Relationships between plasma apoC-III kinetics and metabolic parameters

Strong positive correlations existed between fasting plasma triglycerides and both the apoC-III level (r= 0.83, p<0.001) and secretion rate (r=0.9, p<0.001) in the combined group of obese type 2 diabetic and non-diabetic subjects. However, no significant relationship was observed between the apoC-III fractional catabolic rate and plasma triglycerides (r=0.002; p=0.9), see **Suppl. Figure S1**. The data further reinforce that the secretion rate of apoC-III strongly associates with the concentration of plasma triglycerides. Interestingly, a positive correlation was observed between the apoC-III FCR rate and DNL in $VLDL_1$ (r= 0.55, p<0.01). We also observed a positive correlation between HbA1c and DNL in $VLDL_1$ (r=0.45, p< 0.05) and a negative correlation between insulin and DNL in $VLDL_1$ (r= -0.56, p<0.01). Plasma fasting TRL-cholesterol concentrations correlated positively with plasma apoC-III concentrations (r=0.48, p<0.05), and plasma RLP-cholesterol concentration correlated with the apoC-III secretion rate and apoC-III concentrations (r=0.49, p<0.05 and r=0.54, p<0.01 respectively).

Significant correlations were also found between indices of glycemic control and apoC-III kinetic measures. HbA1C and fasting glucose correlated positively with apoC-III secretion rate (**Figure 1** and **Suppl. Figure 5**). HbA1C (r=0.63, p<0.01), fasting glucose (r=0.47, p<0.05) and insulin (r=0.57, p<0.05), correlated also positively with apoC-III fractional catabolic rate (**Figure 1** and **Suppl. Figure 5**).

DISCUSSION

Here, we report that apoC-III metabolism is significantly perturbed in subjects with type 2 diabetes. The apoC-III secretion rate was markedly higher in subjects with diabetes compared with BMI-

matched non-diabetic subjects. Improved glycemic control with liraglutide therapy reduced significantly apoC-III secretion rate and, thereby, apoC-III levels in type 2 diabetic subjects. These findings suggest that glucose homeostasis is a regulator of apoC-III metabolism, and that the secretion rate of apoC-III seems to be an important driver for the elevation of TRLs in type 2 diabetes.

Several investigators have reported elevation of plasma apoC-III in subjects with type 2 diabetes[12], and we designed our study to gain insight into the dynamics of apoC-III metabolism in this condition. We recruited subjects who were treated with metformin only and had satisfactory glycemic control to avoid marked diabetic hypertriglyceridemia. Both groups of diabetic and non-diabetic men were obese with similar BMI and waist circumference, and plasma triglycerides and apoC-III levels only showed a trend to be elevated in the group with type 2 diabetes. However, in line with previous data[45] we observed a positive correlation between plasma triglycerides and apoC-III levels in the combined cohort as well as in the liraglutide intervention study. Likewise, the changes of triglycerides and TRL responses during OFTT correlated with the observed changes of apoC-III responses highlighting the role of apoC-III as a regulator of not only plasma triglycerides but also atherogenic remnants particles that are known to be increased in diabetic dyslipidemia[12,46]. Several studies have also reported a close correlation between VLDL triglycerides and VLDL apoC-III secretion rates[47-51]. Thus, the data are consistent with the previously observed close relationship between apoC-III and triglyceride and remnant levels at the population level in subjects both with and without type 2 diabetes[3,11,12,31,45,51,52].

One of the aims of our study was to investigate factors that regulate apoC-III secretion rate and/or fractional catabolic rate. Glucose, insulin and free fatty acids are reported to modulate apoC-III expression[13,30]. Carron *et al* reported that both glucose and insulin regulate apoC-III transcription but in opposite ways and they proposed that the dysregulation of apoC-III is a critical factor to explain the dyslipidemia in insulin-resistant states[27]. We observed some positive correlations between measures of glucose homeostasis and apoC-III secretion rate in the cross-sectional study. Furthermore, the changes of glycemic parameters during liraglutide therapy correlated with changes of apoC-III secretion reinforcing the role of glycemic control as a determinant of apoC-III secretion rate. Notably, the effect of glucose on apoC-III expression in hepatocytes has *in vitro* been shown to be mediated by carbohydrate responsive element binding protein and HNF4α genes[30]. Thus, the effects of liraglutide therapy seem to be linked to improved glycemic control. Direct effects of liraglutide on liver cells seem less likely since several studies have failed to confirm the expression of the canonical GLP-1R in hepatocytes[30,53,54]. Therefore, many actions by GLP-1 agonists are likely indirectly mediated, possibly through neural circuits.

Unexpectedly, the apoC-III catabolic rate was also significantly higher in the men with type 2 diabetes in our cross-sectional study. We cannot exclude the possibility that this effect was caused by

the statin therapy leading to increased LDL receptor dependent removal of lipoproteins. However, liraglutide therapy did not influence apoC-III catabolic rate in our intervention study. This finding indicates that the effect of liraglutide on apoC-III secretion rate is probably not explained by statin treatment. Still, the effect of statins on apoC-III metabolism remains to be fully clarified in larger studies[55].

Interestingly, the measures of glycemic control correlated positively with apoC-III clearance rate. The major organ for apoC-III clearance seems to be the kidney, which is responsible for the removal of free (i.e. non-lipoprotein associated) apoC-III molecules[56]. We therefore estimated the glomerular filtration rate in subjects with and without type 2 diabetes but did not find any significant difference, which is not surprising since subjects with kidney disease, were excluded from the study. This raises the question why impaired glycemic control correlates with increased apoC-III clearance? One possibility could be that the different sialylated isoforms of apoC-III have different clearance pathways, as Mauger *et al* have reported different clearance kinetics of the apoC-III isoforms[57]. In line, changes in apoC-III glycoisoform ratios have been observed in kidney diseases[58,59].

The role of apoC-III in atherogenesis has attracted major interest during recent years. The finding that glucose regulates *ApoC3* expression raises the role of apoC-III in cardiovascular risk in diabetes. Diabetic dyslipidemia is characterized by hypertriglyceridemia and accumulation of remnant particles, and apoC-III may aggravate the hypertriglyceridemia by impairing the lipolysis of triglyceride-rich lipoproteins. In addition, our results indicate that apoC-III also affect the removal of remnant particles, resulting in accumulation of atherogenic remnant particles[60,61]. This may explain the improved postprandial lipemia and robust reduction of surrogate markers of remnant particles during liraglutide therapy. Thus, our findings highlight the importance of optimal glycemic control in Type 2 diabetic subjects with dyslipidemia and high cardiovascular risk.

Our study has several strengths. First, the study-design enabled us for the first time to estimate kinetic parameters of apoC-III using stable isotopes in subjects with type 2 diabetes. Second, the quantification of apoC-III enrichment using PRM on a high resolution/accurate mass instrument resulted in high specificity and sensitivity. In earlier studies using stable isotopes to study apoC-III kinetics, only apoC-III_1 was investigated because of its greater concentration in plasma[44]. Notably, the observed correlations were demonstrated using both the cross-sectional cohorts and in the intervention study where the relationship was enforced by the correlations between changes of glycemic parameters and apoC-III secretion rate observed during liraglutide therapy that improved glycemic control.

However, the study has also weaknesses. We examined a small number of study subjects because of the complex methodology for analyzing enrichment of apoC-III. Therefore, we analyzed correlations with the total plasma apoC-III instead of analyzing apoC-III in all lipoprotein fractions. This may be a

limitation given that apoC-III exchanges between different lipoprotein fractions, and this may result in different clearance rates[47]. However, the Mendelian randomization studies that demonstrated the link between apoC-III and CVD were performed using total plasma apoC-III levels[6,7]. In addition, this is a pilot study and the results are hypothesis generating and need to be validated in future studies. The subjects with type 2 diabetes received statin treatment during the study since ethical considerations prohibited us from statin withdrawal during the study. Critically, statin treatment was maintained constant during the study. Statin treatment has been shown to lower plasma apoC-III levels [55,62]. Thus, statin treatment of the type 2 diabetic subjects would likely offset the real differences (i.e., it's predictable that off statins the changes of apoC-III concentrations would have been more pronounced).

In summary, the present study points toward a key role of glycemic control in the metabolism of apoC-III and consequently in the pathophysiology of dyslipidemia in type 2 diabetic subjects. Future studies are important to verify the results and to test the concept that apoC-III synthesis may be an attractive therapeutic target to reduce the residual risk in particular in type 2 diabetic subjects with abnormal TRL metabolism to reduce the burden of CVD.

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DUALITY OF INTEREST

The authors report no duality of interest.

CONTRIBUTION STATEMENT

The authors contributed to the present work as follows: MRT, NM, KP and JB contributed to conception and design, MA, LA, JK, KP, NM, SS, AH, NL, CS, AT and HZ to the acquisition of data or analysis, and MA, MRT, EB, CP and JB to the interpretation of data. MA, MRT, CP and JB

drafted the original and revised manuscripts and all authors approved the final approval of the version to be published.

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FIGURE LEGENDS

Figure 1. Individual apoC-III kinetic parameters before and after liraglutide treatment. (A) apoC-III secretion rate was decreased, (B) apoC-III fractional catabolic rate was unchanged and, (C) the apoC-III pool size was decreased (see Suppl. Table S1 for measured concentration). See Table 1 for mean and SD values. Secretion rate, SR; fractional catabolic rate, FCR. [a]estimated average apoC-III concentration.

Figure 2. Relation between changes in kinetic parameters and changes in glucose and glucose control. (A) apoC-III SR vs plasma glucose, (B) apoC-III pool vs plasma glucose, (C) apoC-III SR vs HbA1c, (D) apoC-III pool vs HbA1C, (E) apoC-III SR vs plasma TG AUC, and (F) apoC-III pool vs plasma TG AUC. Secretion rate, SR; fractional catabolic rate, FCR. [a]estimated average apoC-III concentration. Linear regressions are represented by solid lines. Dashed lines represent 95% CI for regression. SR, Secretion rate; FCR, fractional catabolic rate; AUC, area under the curve; TG, plasma triglycerides.

Figure 3. Correlations between (A) apoC-III secretions rate and (B) apoC-III fractional catabolic rate and apoC-III plasma concentration. Correlations between HbA1c and (C) apoC-III secretions rate and (D) apoC-III fractional catabolic rate in subjects without (●) and with type 2 diabetes (○). Linear regressions are represented by solid lines. Dashed lines represent 95% CI for regression. SR, Secretion rate; FCR, fractional catabolic rate.

Table 1. ApoC-III kinetics in the postprandial period in subjects with type 2 diabetes before and after treatment with liraglutide

	Before liraglutide intervention			During liraglutide intervention			Change	
Kinetic parameters	mean	SD	Range	mean	SD	Range	%	p-value
ApoC-III SR (mg/kg/day)	6.6	1.7	3.9–10.1	5.8	2.0	2.7–10.8	-12.0	**0.030**
ApoC-III SR (mg/day)	652	196	380–1045	561	198	245–1091	-14	**0.022**
ApoC-III FCR (pools/day)	1.2	0.1	1.1–1.5	1.2	0.2	1.0–1.5	2.2	0.397
ApoC-III (mg/dl)[a]	12.4	3.7	6.0–18.7	10.7	3.7	3.9–18.1	-13.1	**0.030**

[a] Estimated average apoC-III concentration; Secretion rate, SR; fractional catabolic rate, FCR.

Table 2. Biochemical measures and apoC-III kinetic parameters in non-diabetic subjects and subjects with type 2 diabetes

	Non-diabetic (n=11)			Type 2 diabetes (n=11)			
	mean	SD	Range	mean	SD	Range	*p*-value
Age, years	58.7	5.6	46–65	60.5	6.1	47–68	0.533
Diabetes duration, years				6.6	4.0	2–15	–
Weight, kg	102.5	11.8	90.6–124.2	100.3	10.5	81.4–114.4	0.818
Waist, cm	111.5	7.1	101.0–124.5	114.1	7.7	102.5–130.5	0.577
BMI, kg/m^2	30.4	2.8	26.7–33.9	31.9	3.7	26.8–37.8	0.309
Liver fat, %	6.06	5.13	0.6–16.7	15.49	7.77	3.7–32.8	**0.004**
Intra–abdominal fat, cm^3	3174	955	1877–4944	3454	1065	2612–6372	0.376
Subcutaneous fat, cm^3	3783	1025	1820–5541	3933	1172	2399–5972	0.670
eGFR, ml/min/1,73 m^2	69.9	3.2	66.3–76.9	68.9	3.5	64.5–76–4	0.224
3–OHB, mg/dl	1.17	0.70	0.6–2.5	1.40	1.30	0.5–4.9	0.718
DNL, µmol/l	5.6	5.3	0–15.7	19.0	8.4	5.8–30.6	**p<0.001**
Glucose homeostasis							
P–glucose, mmol/l	5.66	0.36	5.1–6.3	9.35	2.52	6.5–13.5	**p<0.001**
P–insulin, µIU/mL	10.7	6.5	4.3–25.2	19.2	9.1	10.7–43.6	**p<0.001**
HbA1C, %	5.55	0.25	5.0–5.9	6.89	0.73	6.0–8.3	**p<0.001**
HbA1C, mmol/mol	37.0	2.7	31.0–40.9	52.8	8.0	42.0–67.3	**p<0.001**
Matsuda index	4.6	2.3	2.1–10.4	2.28	140	1.05–5.9	**0.003**
HOMA2-IR	1.41	0.86	0.58–3.36	2.34	0.73	1.06–3.42	**0.02**
Lipids and lipoproteins							
Triglycerides, mmol/l	1.48	0.54	0.7–2.4	1.90	0.73	0.9–3.0	0.200
Cholesterol, mmol/l	5.19	0.68	3.7–6.2	3.89	0.79	2.7–5.5	**0.002**
LDL-C, mmol/l	3.59	0.64	2.6–4.5	2.13	0.79	0.8–3.5	**p<0.001**
HDL-C, mmol/l	1.10	0.41	0.6–2.1	1.13	0.24	0.7–1.5	0.577
TRL-cholesterol, mg/dl	33.5	12.2	12.6–55.0	22.5	8.3	7.4–36.4	**0.023**
RLP-cholesterol, mg/dl	9.1	6.0	2.5–25.1	8.3	3.0	2.3–16.4	0.818
ApoAI, mg/dl	138.6	30.1	86–190	122.4	13.7	99.8–146	0.139
ApoC-III, mg/dl[a]	10.29	2.97	3.5–13.5	11.79	4.70	3.2–17.7	0.140
Kinetic parameters							
ApoC-III SR (mg/kg/day)	4.87	1.34	2.6–6.6	6.75	1.91	3.8–9.7	**0.007**
ApoC-III SR (mg/day)	505	174	266–806	676	208	382–1114	**0.042**
ApoC-III FCR (pools/day)	1.05	0.17	0.76–1.33	1.23	0.16	1.04–1.46	**0.019**
ApoC-III (mg/dl)[b]	10.47	2.80	4.76–14.7	12.58	4.24	5.8–18.6	0.10

Biochemical measures were analyzed in fasting samples, and the apoC-III kinetics in the postprandial period. [a] measured apoC-III concentration at baseline; [b] estimated average apoC-III concentration; Secretion rate, SR; fractional catabolic rate, FCR.

Figure 1

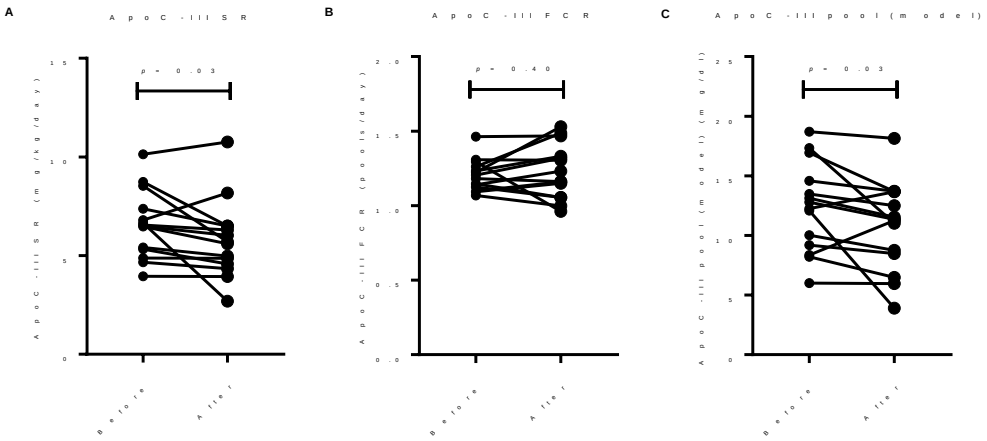

Figure 2

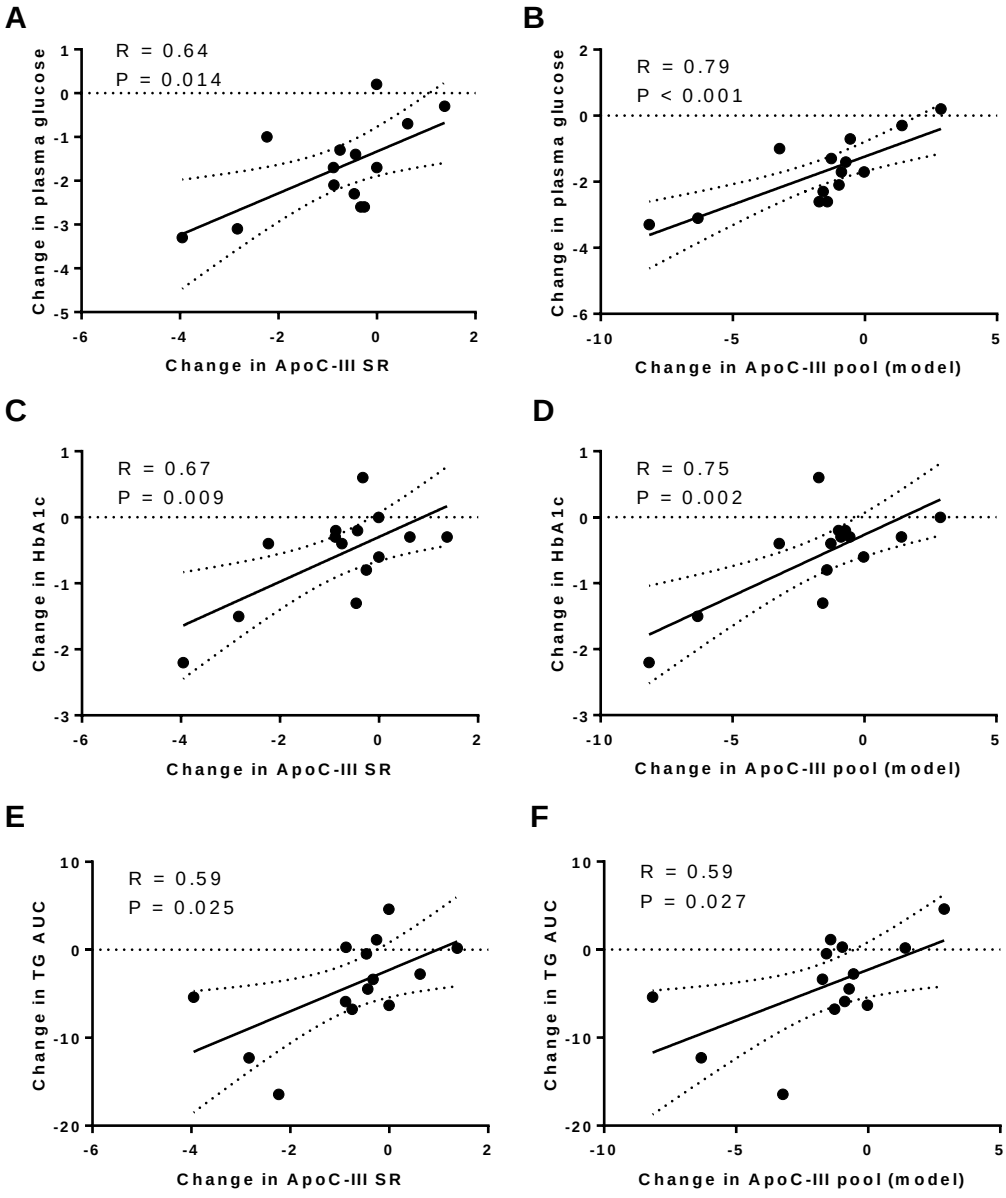

Figure 3